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## The Culture Collection of Algae at Göttingen University (SAG): a biological resource for biotechnological and biodiversity research

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### Abstract

The SAG is one of the most comprehensive resources of microalgal cultures ([www.epsag.uni-goettingen.de](http://www.epsag.uni-goettingen.de)). It is supporting research in biotechnology and biodiversity through *ex situ* conservation of algae and expert knowledge on identifying and isolating. Multiple strains proven to represent the same microalgal species exhibit extensive genotypic diversity interesting for further exploitation. Cryopreservation is well suited to circumvent problems associated with perpetual maintenance, but needs optimization to ensure genetic stability. To ensure the SAG's reliability, primary goals are correctly identified strains as references for DNA sequence comparisons. Novel isolates from unusual terrestrial habitats worth further biotechnological exploitation are being developed.

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### 1. Introduction

The SAG culture collection of algae is a comprehensive biological resource of living algae; it is among the three largest algal service culture collections in the world. It is striving to provide targeted service and support to workers involved in biotechnological applications of microalgae as well as it serves the scientific and educational communities worldwide. With their main functions of preserving and providing

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algal resources, algal culture collections serve an essential infrastructural function for scientific investigation. Algal culture collections contribute as resource and service infrastructure as well as repository of expert knowledge on identifying, isolating, culturing and *ex situ* conservation of algae [1]. Although algal culture collections provide an enormous diversity of microalgae, until now only a very minor fraction of the available strain diversity has been exploited for biotechnological purposes. Despite the SAG's commitment to provide the user community with a diversity of algal strains as broad as possible, it put an emphasis on algae from terrestrial (e.g. soil and air-exposed substrates) and freshwater habitats. The reliability of the algal collections' biological resources is essential for research, i.e. to enable reproducibility that is required by any scientific enquiry and biotechnological application. Without culture collections, every user would have to “reinvent the wheel” and invest innumerable hours in the costly recovery of organisms and genes and their characterization [1]. By providing an element of stability and continuity in scientific work and experimental research, the culture collections are indispensable for the progress of algal culture technology. The SAG is also a member of the European Culture Collections Organization (ECCO) and the World Federation of Culture Collections (WFCC).

### *1.1. The SAG as a service culture collection*

As one of the world-wide largest service culture collection, the SAG receives an average of about 600 orders per year corresponding to an average total of about 2100 dispatched cultures (averages of last five years) and both numbers are continuously increasing since 2006. Users are from the scientific and biotechnology communities world-wide. International dispatches increased from 30 % to 47 % within the last five years with about one third of all orders coming from within Europe. About 67% of all orders per year pertains to academia, more than half of this percentage serves education at universities and schools within Germany. Over the last five years there was an increasing demand for SAG's cultures, i.e. about one fifth of all orders came from industry, mainly due to the growing diversity of algal strains used for biotechnological purposes. Despite these numbers may already indicate international importance of the SAG culture collection, it remains impossible to estimate the value of a culture collection by the numbers of orders received per year, because already just a single culture strain could result in a significant scientific insight.

### *1.2. The SAG as a research culture collection*

Besides its service function and being an essential biological resource, the SAG is also actively participating in research. Current research topics to which the SAG is linked include biodiversity analysis of various habitats of terrestrial algae, the isolation of biofouling microalgae and their subsequent utilization as antifouling test-organisms, and screening for commercial metabolites with expert input into process development and up-scaling. The SAG collaborates extensively with the CCAP [2], [3]. A previous EU-funded research project which had the key objective to use cryopreservation to produce a pan-European virtual Biological Resource Centre, linked the SAG with the CCAP and three other European culture collections [4], [5].

### *1.3. The SAG's history in brief*

The SAG was initiated when Professor E.G. Pringsheim (1881-1970), one of the pioneers in algae cultivation, arrived at Göttingen, Germany, in 1953 after his time as a World War II refugee scientist in England [6], [7]. Pringsheim established a first centre of algae cultivation at Charles University in Prague already in the 1920's [6], and this early collection formed the nucleus of three more large service culture

Table 1. Distribution of the 2291 strains currently available in the SAG Culture Collection on Cyanobacteria, major clades of eukaryotes [9], their phyla and classes

Taxonomic groups			no. of strains
Cyanobacteria			230
Archaeplastida	Glaucophyta		14
		Chlorophyta	1025
		Chlorophyceae	269
		Trebouxiophyceae	54
		Ulvophyceae	17
		Prasinophytes	177
	Streptophyta		90
	Rhodophyta		1
Rhizaria	Chlorarachniophyta		161
Excavates	Euglenoids		24
Chromalveolates	Stramenopiles	Bacillariophyceae	110
		Xanthophyceae	22
		Eustigmatophyceae	20
		Phaeophyceae	23
		Chryso- + Synurophyceae	2
		Raphidophyceae	2
		Phaeothamniophyceae	12
		Haptophyta	27
		Cryptophyta	11
		Alveolates	2291
		Dinophyta	

collections, now the Culture Collection of Algae and Protozoa (CCAP), Culture Collection of Autotrophic Organisms (CCALA), and the Collection of Algae at the University of Texas at Austin, UTEX [8]. Today, 304 strains (56 authentic strains, see below) isolated by E.G. and his wife Olga Pringsheim are still available from the SAG. In the intervening years the SAG has rapidly developed and expanded through the enthusiastic curatorial work of Drs W. Koch and U.G. Schlösser. Since 1999 the curatorial duties have been taken over by M. Lorenz with T. Friedl as the director of the SAG.

## 2. Diversity of microalgal strains available from the SAG culture collection and its exploitation

At present (March 2011) 2291 strains of mainly microscopic algae including cyanobacteria are available from the SAG in the public domain. This comprises 538 genera and 1424 named species of algae with 370 additional strains that are still unidentified at the species level. The diversity of algal strains available from the SAG reflects almost all phyla and classes of eukaryotic algae, but it also houses a respectable diversity of cyanobacteria (Table 1).

Cyanobacteria form the second largest focal point of the strain diversity with about 10% of all SAG strains. All four of the five major groups of eukaryotes from a phylogeny that has recently emerged from the synthesis of various information [9] and on which algae are scattered, are well represented in the SAG (Table 1). The “Archaeplastida” clade comprising exclusively eukaryotes with plastids derived from primary endosymbiosis, is best represented in the SAG. Green algae of the phylum Chlorophyta form the largest group in the SAG with almost 60 % of all strains, green algae of the phylum Streptophyta comprise

about 7 % of all SAG strains (Table 1). The latter is mainly represented by the microscopic Klebsormidiophyceae (incl. 30 strains of *Klebsormidium*) and Conjugatophyceae (30 genera), but no Charales are maintained. For the red algae (Rhodophyta; about 4 % of all strains) a considerable number of freshwater representatives (12 genera in 29 strains) and unicellular forms from soils (e.g., *Porphyridium*) or extremely acid environments (*Cyanidium*, *Galdieria*) are maintained. About 11 % (253 strains; Table 1) of the SAG's holdings represent the “Chromalveolates” which comprises large and abundant algal groups with plastids derived from a red algal secondary endosymbiosis [9]. From the Chromalveolates the heterokont algae (Stramenopiles, about 9 % of the SAG holding) are best represented in the SAG, whereas only very few and mainly freshwater forms of dinoflagellates (Alveolates) are available (Table 1). Two smaller groups of heterokont algae that are particularly abundant in terrestrial habitats, i.e. the Xanthophyceae and Eustigmatophyceae, are with about 5 and 1 % of all strains well represented in the SAG. However, the diatoms forming a very large and important group of terrestrial algae are with just 1 % by far not adequately represented. The cryptomonads are represented by a considerable variety of mainly freshwater forms, whereas the haptophytes, a mainly marine group, is only little represented in the SAG. The euglenoids, forming the single algal lineage of the “Excavates” clade [9], form with about 7 % the third largest focal point of the SAG's algal diversity.

The potential of the large diversity of microalgal strains already available in the public domain for applied research has by far not yet been explored. The SAG's microalgae represent an attractive resource of primary and secondary metabolites. Moreover, new secondary metabolites may be a valuable resource, since often they have beneficial properties for medical or nutritional use. Only recently through collaborative efforts projects have been started that aim at screening of SAG strains for valuable compounds and to explore their potential for other algae-based resources, e.g. the production of biomass and capture of CO<sub>2</sub> from industrial flue gases. The first comprehensive analysis that was recently finished, investigated the fatty acid composition of more than 2000 microalgal strains from the SAG in the stationary phase [10].

### 3. Strain identification using reference and authentic strains

A serious concern of service culture collections like the SAG is to provide correctly identified strains. Most identified SAG strains have been provided by specialists who asked or have been asked for accession of their identified material by the SAG. However, despite all efforts to provide the user with correct identifications, the number of strains with unclear species identification is with 16 % still considerable. This is a notorious problem in microalgae because their distinguishing morphological features are often very scant. DNA sequence comparison using conserved marker molecules is a very useful alternative, but it requires a large number of reference sequences from reliably identified strains. A considerable fraction of 37 % of the SAG strain holdings may already serve as reference strains, because conserved marker molecules (mostly nuclear-encoded SSU rRNA or plastid-encoded *rbcL* gene sequences) have been sequenced for them and the sequences are available from the public databases. The proportion of strains for which SSU rRNA or *rbcL* gene sequences have become available is particularly high (about 60 %) in the SAG's Xanthophyceae and euglenoid strains, is about 50 % for all heterokonts (other than Xanthophyceae), about 40 % of all green algal (Chlorophyta and Charophyta) and about 30 % of all cyanobacteria strains. For unambiguous identification at the level of species (e.g., in DNA barcoding) the nuclear-encoded ITS -2 region has successfully been used in green algae, e.g. [11], [12], and diatoms, e.g. [13], and these recent works included already a respectable number of SAG strains. In Xanthophyceae the plastid-encoded *psbA/rbcL* spacer has been found very useful for species delineation [14]. For about 230 (68 authentic) SAG green algal strains (15 %) ITS2 sequences are already available from the public databases, and *psbA/rbcL* spacer for about half of all of Xanthophyceae strains. Currently,

a rapid PCR-based molecular approach which includes ITS2 sequencing is being developed at SAG as a quality control to unambiguously trace contaminations to which algal cultures especially at larger scales as used in biotechnological applications are exposed. With respect to provide reliable references for strain identification, the large number of so-called authentic strains available from the SAG is of pivotal importance. Authentic strains have been derived from type material on which the formal taxonomic description of an algal species has been based. Their value for taxonomy is even greater if cultures of authentic strains are kept in cryogenic storage [3]. A total of 467 SAG strains (20 %) are authentic with a particularly high percentage in three taxonomic groups: about one fourth of all SAG's Chlorophyta and Xanthophyceae strains are authentic, in Eustigmatophyceae even about half of the strains. For about 50 % of all SAG authentic strains SSU rRNA or *rbcL* gene sequences are yet available in public databases.

#### 4. Multiple and duplicate strains per species: genotypic diversity

There are 280 named species of microalgae provided by the SAG which are represented by multiple strains, many of them of high interest for applied research. There are even 40 species, mainly from green algae, which are represented by 5 or more strains per species, for example *Euglena gracilis* (31), *Chlamydomonas moewusii* (31), *C. reinhardtii* (18) and *Chlorella vulgaris* (15). Recent genotypic and biochemical analyses were in favour of valuable genetic and phenotypic diversity rather than redundancy, exhibited by multiple strains. When representing isolates of different origins, i.e. from geographically distant localities or different ecological niches, genetic diversity may be expected for the multiple strains of a species [15]. Therefore, a serious concern of the SAG is to ensure conspecificity and to detect unique genotypes among the multiple strains per species. As an example, sequence comparisons of ITS rDNA have assessed conspecificity of the 15 SAG strains named *Chlorella vulgaris* with the original Beijerinck isolate, SAG 211-11b, which is the authentic strain of the species, kept in pure culture already since more than 110 years [16]. AFLPs, a highly sensitive fingerprinting method for the simultaneous analysis of multiple loci spread over of the whole genome, unraveled considerable genetic variation among the 15 strains which was interpreted as corresponding to at least five "hidden" (cryptic) species in *C. vulgaris* [15].

A considerable number of the same algal clone as represented in the SAG culture collection is shared with other culture collections (duplicate strains), previously done for reasons of preventing accidental loss or contamination and ensuring ready worldwide availability of important strains. As an example, 84 strains that have been isolated by the SAG's founder, E.G. Pringsheim, are still available in triplicate among the SAG, CCAP and UTEX culture collections which reflects their historic links [8]. Despite duplicate strains represent the same clonal isolate, an "artificial" diversity may have been introduced by different maintenance methods and culture regimes at the various collections over decades which may have led to differences in selective pressures and/or genetic drift. Also, over many decades human error may result in a higher likelihood of mislabeling or a "mix up" on performing a serial transfer [2]. For the ten duplicate strains of *Chlorella vulgaris* shared among the SAG, CCAP and UTEX collections, highly sensitive AFLP fingerprints resolved pairs of duplicate strains in all except two cases. The latter could be explained by one mix up and one erroneous exchange for a more distantly related species [15]. Obviously, there is always the risk of human error, and this is a clear disadvantage of the traditional maintenance of microalgal cultures in an actively metabolizing state [2], [17].

## 5. Cryopreservation and testing for genetic stability

There is a clear need for genetically stable cultures of algae for use in biotechnology. The most common method for maintenance of algal cultures still is serial subculturing of actively growing cultures under suboptimal conditions [2], at the SAG as well as most other service culture collections. However, continuous subculturing over thousands of generations under suboptimal conditions undoubtedly affects algal strains in various ways and it is well accepted that the genetic and phenotypic stability of algal strains cannot be guaranteed over years of routine maintenance [17]. Cryopreservation, i.e. the storage of the cells at an ultralow temperature [18] is a very promising alternative that may also reduce the risk of contaminations and "mix ups", because after the initial cryopreservation, no regular handling procedures are involved [17]. At SAG, so far 549 out of 743 tested strains have successfully been cryopreserved with reasonable viability as tested in growth experiments after thawing. In the Chlorophyta with 78% of 552 tested SAG strains the rate of successfully cryopreserved strains was very high, whereas in the SAG's strain holdings for Chromalveolates the success rate was very low. We observed that the unicellular terrestrial green algae isolated from air-exposed substrates, e.g. those from green biofilms covering man-made substrates, could be cryopreserved with an almost 100 % success rate using a standard two-step protocol.

The risk of genomic alteration is fundamental to any preservation procedure particularly where protocols are not optimised [19]. The cryopreservation process itself may induce various stresses, cryoprotectants and/or oxidative stress may cause the formation of free radicals which may cause genetic alterations [20], [21]. To assess genetic stability, AFLP fingerprint patterns were generated at the SAG culture collection before and after cryopreservation to assess genetic stability in selected strains of microalgae, i.e. green algae [19], euglenoids [22] and some cyanobacteria (K. Sauer, unpubl).

In a study of various green algae no differences were found between AFLP patterns before and after cryopreservation in only 10 out of 24 tested strains, the others were clearly differing in their respective patterns [19]. Half of the latter strains had AFLP pattern differences in the same range ( $> 1.8\%$ ) as those found for two phenotypically distinct mutants of *Parachlorella kessleri* compared to their wildtype [19]. The observed AFLP pattern differences after thawing could indicate that the standard two-step cooling protocol may be suboptimal for some strains. AFLP differences observed in cryopreservation of euglenoids may be attributed to progressive osmotic stress caused during vitrification [22]. The AFLP method is sensitive to higher levels of DNA methylation which may be caused in the algal cells by stress. AFLPs may therefore be useful as a means of evaluating the effectiveness of cryopreservation protocols.

## 6. Novel isolates with high potential for applied research

An additional resource that should receive attention with respect to biotechnological exploitation is a constantly growing collection of new unique isolates provided by the SAG's linkage to current research projects. They primary focus on an assessment of algal diversity in unusual terrestrial habitats for mainly three reasons: One is to better understand phylogenetic relationships of the still poorly investigated terrestrial algae and to recover new species, another is to test and develop new methods for assessing algal diversity in a given habitat as complete as possible. Finally, algal communities from biofilms and in soils are compared along gradients of certain abiotic factors to better understand the ecological preferences of terrestrial algae. Despite verification by biochemical analyses and growth experiments are still missing, the significant value of the new terrestrial isolates may already be anticipated from the observation of their pigmentation and morphological traits such as presence of lipid droplets, remarkable growth characteristics, and their close phylogenetic relatedness to already exploited species. Despite terrestrial algae were often dwelling in extreme and rather dry environments, the great majority of the new isolates



has been found to grow well under the SAG's standard culture conditions (18 or 20 °C under 20-50  $\mu$ E and a light/dark cycle of 12:12 hrs; [2]). In liquid culture media they even reached higher densities than their counterparts from temperate environments and were more robust than cultures of phytoplankton species. Many of the new isolates were found also resistant to shaking and air bubbling (which is an essential prerequisite to be used in applied research) and, therefore, could be scaled up to volumes larger than test tubes. Novel isolates currently being studied include isolates from soils from Antarctica, hypersaline soils (Solochank) in the south of Ukraine, biofilms covering rocks in a high carbonate freshwater environment, including sites exhibiting very high CO<sub>2</sub> partial pressures [23], biofouling green algae causing biodeterioration on building material [24] as well as biological soil crusts in Southern Africa [25].

We anticipate sharing the new isolates as well as the whole current SAG's holdings of algal and cyanobacterial strains in collaborative efforts with the biotechnological community in order to screen for valuable compounds and investigate their further exploitation.

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